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                    and display fields
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 NEWS 13   JUl 11   CHEMSAFE reloaded and enhanced
 NEWS 14   JUl 14   FSTA enhanced with Japanese patents
 NEWS 15   JUl 19   Coverage of Research Disclosure reinstated in DWPI
 NEWS 16   AUG 09   INSPEC enhanced with 1898-1968 archive

 NEWS EXPRESS  JUNE 30 CURRENT WINDOWS VERSION IS V8.01b, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 26 JUNE 2006.

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 NEWS LOGIN    Welcome Banner and News Items
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  * The files listed above are temporarily unavailable.

FILE 'HOME' ENTERED AT 11:12:49 ON 28 AUG 2006

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MOST RECENT UPDATE WEEK:        200633        <200633/EW>
FILE COVERS 1978 TO DATE

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    PLEASE SEE HELP COST <<<

=> s HPMA
           533 HPMA
             2 HPMAS
L1         535 HPMA
               (HPMA OR HPMAS)
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=> s dox?
L2       17624 DOX?

=> s l2 and l1
L3         216 L2 AND L1

=> s antibod?
L4       90525 ANTIBOD?

=> s cancer? or neoplas? or tumor?
         80813 CANCER?
         23424 NEOPLAS?
         67406 TUMOR?
L5      100537 CANCER? OR NEOPLAS? OR TUMOR?

=> s l3 and l4 and l5
L6         191 L3 AND L4 AND L5

=> s l6 not py>1999
        716878 PY>1999
L7          14 L6 NOT PY>1999

=> d ibib 1-14

L7     ANSWER 1 OF 14    PCTFULL   COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER:        1999049901 PCTFULL  ED 20020515
TITLE (ENGLISH):         WATER SOLUBLE PACLITAXEL DERIVATIVES
TITLE (FRENCH):          DERIVES DE PACLITAXEL SOLUBLES DANS L'EAU
INVENTOR(S):             LI, Chun;
                         WALLACE, Sidney;
                         YU, Dong-Fang;
                         YANG, David
PATENT ASSIGNEE(S):      PG-TXL COMPANY, L.P.;
                         LI, Chun;
                         WALLACE, Sidney;
                         YU, Dong-Fang;
                         YANG, David
LANGUAGE OF PUBL.:       English
DOCUMENT TYPE:           Patent
PATENT INFORMATION:
                         NUMBER              KIND     DATE
                         ---------------------------------
                         WO 9949901            A1 19991007
DESIGNATED STATES
       W:                AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK
                         EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP
                         KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL
                         PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN
                         YU ZA ZW GH GM KE LS MW SD SL SZ UG ZW AM AZ BY KG KZ
                         MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU
                         MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD
                         TG
APPLICATION INFO.:       WO 1999-US6870       A  19990330
PRIORITY INFO.:          US 1998-09/050,662      19980330

L7     ANSWER 2 OF 14    PCTFULL   COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER:        1999007324 PCTFULL  ED 20020515
TITLE (ENGLISH):         CONJUGATES TARGETED TO THE INTERLEUKIN-2 RECEPTOR
TITLE (FRENCH):          CONJUGUES CIBLES APPORTES AU RECEPTEUR DE
                         L'INTERLEUKINE-2
INVENTOR(S):             PRAKASH, Ramesh, K.
PATENT ASSIGNEE(S):      THERATECH, INC.
LANGUAGE OF PUBL.:       English
DOCUMENT TYPE:           Patent
```

PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9907324	A2	19990218

DESIGNATED STATES

W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

APPLICATION INFO.: WO 1998-US16290 A 19980805
PRIORITY INFO.: US 1997-08/914,042 19970805

L7 ANSWER 3 OF 14 PCTFULL COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER: 1998056425 PCTFULL ED 20020514
TITLE (ENGLISH): PHARMACEUTICAL COMPOSITIONS CONTAINING ANTIBODY -ENZYME CONJUGATES IN COMBINATION WITH PRODRUGS
TITLE (FRENCH): COMPOSITIONS PHARMACEUTIQUES CONTENANT DES CONJUGUES ANTICORPS-ENZYMES COMBINES A DES PROMEDICAMENTS
INVENTOR(S): DUNCAN, Ruth;
SATCHI, Ronit
PATENT ASSIGNEE(S): THE SCHOOL OF PHARMACY, UNIVERSITY OF LONDON;
DUNCAN, Ruth;
SATCHI, Ronit
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9856425	A1	19981217

DESIGNATED STATES

W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG

APPLICATION INFO.: WO 1998-GB1700 A 19980611
PRIORITY INFO.: GB 1997-97304070.2 19970611

L7 ANSWER 4 OF 14 PCTFULL COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER: 1998051336 PCTFULL ED 20020514
TITLE (ENGLISH): TARGETED DELIVERY TO T LYMPHOCYTES
TITLE (FRENCH): ADMINISTRATION CIBLEE VERS DES LYMPHOCYTES T
INVENTOR(S): PRAKASH, Ramesh, K.;
KUMAR, Vijay
PATENT ASSIGNEE(S): THERATECH, INC.
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9851336	A1	19981119

DESIGNATED STATES

W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG

APPLICATION INFO.: WO 1998-US9057 A 19980504
PRIORITY INFO.: US 1997-8/857,009 19970515

L7 ANSWER 5 OF 14 PCTFULL COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER: 1998035684 PCTFULL ED 20020514
TITLE (ENGLISH): METHODS FOR DETECTION OF KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS-LIKE VIRUS
TITLE (FRENCH): METHODES DE DETECTION D'UN VIRUS SEMBLABLE A L'HERPESVIRUS ASSOCIE AU SARCOME DE KAPOSI
INVENTOR(S): BERENSON, James, R.;
RETTIG, Matthew, B.;
VESCIO, Robert, A.
PATENT ASSIGNEE(S): BERENSON, James, R.;
RETTIG, Matthew, B.;
VESCIO, Robert, A.
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9835684	A2	19980820

DESIGNATED STATES
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
APPLICATION INFO.: WO 1998-US2820 A 19980212
PRIORITY INFO.: US 1997-8/800,710 19970214
US 1997-8/967,504 19971111

L7 ANSWER 6 OF 14 PCTFULL COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER: 1997033618 PCTFULL ED 20020514
TITLE (ENGLISH): TARGETING MACROMOLECULAR PRODRUGS TO T LYMPHOCYTES
TITLE (FRENCH): PROMEDICAMENTS MACROMOLECULAIRE DE CIBLAGE DES LYMPHOCYTES T
INVENTOR(S): PRAKASH, Ramesh, K.;
KOPECEK, Jindrich;
KOPECKOVA, Pavla;
OMELYANENKO, Vladimir
PATENT ASSIGNEE(S): THERATECH, INC.;
UNIVERSITY OF UTAH RESEARCH FOUNDATION
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9733618	A1	19970918

DESIGNATED STATES
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG UZ VN YU GH KE LS MW SD SZ UG AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
APPLICATION INFO.: WO 1997-US3832 A 19970312
PRIORITY INFO.: US 1996-8/616,693 19960315

L7 ANSWER 7 OF 14 PCTFULL COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER: 1997015285 PCTFULL ED 20020514
TITLE (ENGLISH): NON-POLYMERIC SUSTAINED RELEASE DELIVERY SYSTEM

TITLE (FRENCH): SYSTEME D'ADMINISTRATION A LIBERATION PROLONGEE NON POLYMERE
INVENTOR(S): DUNN, Richard, L.
PATENT ASSIGNEE(S): ATRIX LABORATORIES, INC.;
DUNN, Richard, L.
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9715285	A1	19970501

DESIGNATED STATES
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG US UZ VN KE LS MW SD SZ UG AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
APPLICATION INFO.: WO 1996-US17082 A 19961025
PRIORITY INFO.: US 1995-8/549,414 19951027

L7 ANSWER 8 OF 14 PCTFULL COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER: 1995027481 PCTFULL ED 20020514
TITLE (ENGLISH): LIQUID DELIVERY COMPOSITIONS
TITLE (FRENCH): COMPOSITIONS LIQUIDES A DIFFUSION
INVENTOR(S): YEWEY, Gerald, L.;
KRINICK, Nancy, L.;
DUNN, Richard, L.;
RADOMSKY, Michael, L.;
BROUWER, Gerbrand;
TIPTON, Arthur, J.
PATENT ASSIGNEE(S): ATRIX LABORATORIES, INC.
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9527481	A1	19951019

DESIGNATED STATES
W: AM AT AU BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU IS JP KE KG KP KR KZ LK LR LT LU LV MD MG MN MW MX NL NO NZ PL PT RO RU SD SE SG SI SK TJ TM TT UA UG UZ VN KE MW SD SZ UG AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
APPLICATION INFO.: WO 1995-US3792 A 19950327
PRIORITY INFO.: US 1994-8/225,140 19940408

L7 ANSWER 9 OF 14 PCTFULL COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER: 1995015770 PCTFULL ED 20020514
TITLE (ENGLISH): PRETARGETING METHODS AND COMPOUNDS
TITLE (FRENCH): PROCEDES ET COMPOSES DE PRECIBLAGE
INVENTOR(S): GRAVES, Scott, S.;
BJORN, Michael, J.;
RENO, John, M.;
AXWORTHY, Donald, B.;
FRITZBERG, Alan, R.;
THEODORE, Louis, J.
PATENT ASSIGNEE(S): NEORX CORPORATION
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE

	WO 9515770 A1 19950615
DESIGNATED STATES	
W:	CA JP AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
APPLICATION INFO.:	WO 1994-US14223 A 19941209
PRIORITY INFO.:	US 1993-8/164,302 19931209

L7 ANSWER 10 OF 14 PCTFULL COPYRIGHT 2006 Univentio on STN

ACCESSION NUMBER:	1995012385 PCTFULL ED 20020514
TITLE (ENGLISH):	MICROPARTICULAR PHARMACEUTICAL COMPOSITIONS IN MICELLAR FORM
TITLE (FRENCH):	COMPOSITIONS PHARMACEUTIQUES MICROPARTICULAIRES SOUS FORME MICELLAIRE
INVENTOR(S):	CHO, Young, W.
PATENT ASSIGNEE(S):	ISOTECH MEDICAL, INC.; CHO, Young, W.
LANGUAGE OF PUBL.:	English
DOCUMENT TYPE:	Patent
PATENT INFORMATION:	

NUMBER	KIND	DATE
WO 9512385	A1	19950511

DESIGNATED STATES	
W:	CA JP KR US AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
APPLICATION INFO.:	WO 1994-US12351 A 19941103
PRIORITY INFO.:	US 1993-8/146,747 19931103

L7 ANSWER 11 OF 14 PCTFULL COPYRIGHT 2006 Univentio on STN

ACCESSION NUMBER:	1994017829 PCTFULL ED 20020513
TITLE (ENGLISH):	DIRECTED BIODISTRIBUTION OF SMALL MOLECULES
TITLE (FRENCH):	BIODISTRIBUTION DIRIGEE DES PETITES MOLECULES
INVENTOR(S):	GUSTAVSON, Linda, M.; FRITZBERG, Alan, R.
PATENT ASSIGNEE(S):	NEORX CORPORATION
LANGUAGE OF PUBL.:	English
DOCUMENT TYPE:	Patent
PATENT INFORMATION:	

NUMBER	KIND	DATE
WO 9417829	A1	19940818

DESIGNATED STATES	
W:	CA JP AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
APPLICATION INFO.:	WO 1994-US1036 A 19940126
PRIORITY INFO.:	US 1993-8/012,533 19930202

L7 ANSWER 12 OF 14 PCTFULL COPYRIGHT 2006 Univentio on STN

ACCESSION NUMBER:	1993014142 PCTFULL ED 20020513
TITLE (ENGLISH):	DRUG DELIVERY SYSTEM FOR THE SIMULTANEOUS DELIVERY OF DRUGS ACTIVATABLE BY ENZYMES AND LIGHT
TITLE (FRENCH):	SYSTEME D'APPORT DE MEDICAMENTS POUR L'APPORT SIMULTANE DE MEDICAMENTS ACTIVABLES PAR LES ENZYMES ET LA LUMIERE
INVENTOR(S):	KOPECEK, Jindrich; KRINICK, Nancy
PATENT ASSIGNEE(S):	UNIVERSITY OF UTAH
LANGUAGE OF PUBL.:	English
DOCUMENT TYPE:	Patent
PATENT INFORMATION:	

NUMBER	KIND	DATE
WO 9314142	A1	19930722

DESIGNATED STATES	
W:	AU BB BG BR CA CZ DE FI HU JP KP KR LK MG MN MW NO PL RO RU SD SK AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE

APPLICATION INFO.: WO 1993-US683 A 19930121
PRIORITY INFO.: US 1992-7/822,924 19920121

L7 ANSWER 13 OF 14 PCTFULL COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER: 1993013804 PCTFULL ED 20020513
TITLE (ENGLISH): PHARMACEUTICAL COMPOSITIONS CONTAINING POLYMER DERIVATIVE-BOUND ANTHRACYCLINE GLYCOSIDES AND A METHOD FOR THEIR PREPARATION
TITLE (FRENCH): COMPOSITIONS PHARMACEUTIQUES CONTENANT DES GLYCOSIDES D'ANTHRACYCLINE LIES A DES DERIVES POLYMERES, ET LEUR PROCEDE DE PREPARATION
INVENTOR(S): ADAMI, Marco;
MAGRINI, Roberto;
MARANGHI, Paolo;
SUARATO, Antonino
PATENT ASSIGNEE(S): FARMITALIA CARLO ERBA SRL
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9313804	A1	19930722

DESIGNATED STATES
W: AU CA FI HU JP KR NZ RU UA AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
APPLICATION INFO.: WO 1992-EP2968 A 19921221
PRIORITY INFO.: GB 1992-9200247.6 19920107

L7 ANSWER 14 OF 14 PCTFULL COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER: 1992010212 PCTFULL ED 20020513
TITLE (ENGLISH): ANTHRACYCLINE-CONJUGATES
TITLE (FRENCH): CONJUGUES D'ANTHRACYCLINE
INVENTOR(S): ANGELUCCI, Francesco;
RUGGIERI, Daniella;
STEFANELLI, Stefania;
SUARATO, Antonino;
BERSANI, Laura
PATENT ASSIGNEE(S): FARMITALIA CARLO ERBA SRL;
ANGELUCCI, Francesco;
RUGGIERI, Daniella;
STEFANELLI, Stefania;
SUARATO, Antonino;
BERSANI, Laura
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9210212	A1	19920625

DESIGNATED STATES
W: AT AU BE CA CH DE DK ES FI FR GB GR HU IT JP KR LU MC NL SE SU US
APPLICATION INFO.: WO 1991-EP2284 A 19911203
PRIORITY INFO.: GB 1990-9026491.2 19901205

=> d kwic 14

L7 ANSWER 14 OF 14 PCTFULL COPYRIGHT 2006 Univentio on STN

DETD Anthracycline-Conjugates
The present invention relates to conjugates of therapeutically useful anthracyclines with carriers such as polyclonal and monoclonal antibodies or proteins or peptides

of natural or synthetic origin; methods for their preparation, pharmaceutical composition containing them and use thereof in treating certain mammalian tumors. The invention also relates to new anthracycline derivatives and their preparations
In the last years? many highly cytotoxic anthracyclines have been synthesized. For example,. . . a morpholino or morpholino substituted ring linked at C-31 position of the sugar moiety have shown promising antitumor activity on experimental murine tumors [see.

The scope of the present invention is to provide anthracycline conjugates with carriers such as monoclonal or polyclonal antibodies or proteins or peptides or other carriers of synthetic origin in order to take advantage of the high potency of the anthracyclines,. . .

The carrier is typically selected from a polyclonal antibody, or fragment thereof comprising an antigen binding site, capable of binding to a tumor associated antigen; a monoclonal antibody, or fragment thereof comprising an antigen binding site, capable of binding to an antigen preferentially or selectively expressed on tumor cell populations; a peptide or protein capable of preferentially or selectively binding to a tumor cell; and a polymeric carrier.

The carrier portion T-NH, or T-[COOH). of the conjugates is therefore preferably derived from from polyclonal antibodies raised against tumor associated antigens; or from monoclonal antibodies binding to antigens preferentially or selectively espressed on tumor cell populations; or from natural or recombinant peptides or proteins or growth factors pref erentially or selectively binding to tumor cells; or from natural or synthetic polymeric carriers such as polylysine, polyglutamic acid, polyaspartic acid and their analogues and derivatives, or such as dextran or other polymeric carbohydrate analogues and their derivatives; or from synthetic copolymers such as those derived from N](2-hydroxypropyl)methacrylamide (HPMA) [see: J.Kopecek, Macromolecules. H.Benoit & P.Rempp, Ed.: 505-520 (1982) Pergamon Press. oxford, England); or from poly(aminoacid) copolymers such as poly(GluNatAla,Tyr) which are useful. . . portion may be also derived from portions of the above mentioned carriers, such as the Fab or the F(abl),, fragments of the antibodies, or such as portions of the above mentioned peptides or proteins obtained through recombinant DNA techniques.

Representative examples of the above mentioned antibodies and of respective possible therapeutic applications are.

-anti T-cell antibody T101 [Royston,I. et al., J.Immunol.

1980, 125, 7251
-anti CD5 antibody OKT1 (Ortho) ATCC CRL 8000 (cronic lymphocitic leukemias)
-anti trasferrin receptor antibody OKT9 (Ortho) ATCC CRL 8021 (ovaric and other tumors)
-anti melanoma antibody MAb 9 27 (Bumol,T.F. et al., Proc.Natl.Acad.Sci.USA 1982, 79, 1245) (melanomas)

-anti carcinoma markers antibody such as.

. . .

1116 NS-3d ARCC CRL 8019
-anti alpha-fetoprotein OM 3 1 ATCC HB 134 (also hepatomas)
-791T/36 [Embleton, M, J. et al., Br. J. Cancer 1981, 430 5821 (also osteogenic sarcoma)
-B 72o3 [U.S. Pat, No. 4,522,918 (1985)) (colorectal carcinomas and other tumors)
-anti ovarian carcinoma antibody OVB 3 ATCC HB 9147
-anti breast carcinoma antibody (HMGP antigen)
[Aboud]Pirak, E. et al., Cancer Res. 1988, 48, 3188]
]anti bladder carcinoma 1G3.10 [Yu,D.S. et al., Eur.J.Urol.

. . .

recombinant origin are
FGFI EGFr PDGFj TGF]cLr cL]MS, Interleukines, Interferones, TNr, melanotropin (MSH), etc*
The carrier T]CHO is preferably derived from polyclonal or monoclonal antibodies having the carbohydrate moiety, preferentially located in the Fc region, selectively oxidized to aldehyde groups by means of chemical or enzymatic methods,, as. . .

For example, conditions for the condensation (b) (i) between the compounds of formula 5 and antibodies T]NE2 are.

aqueous 0.114 sodium phosphate and aqueous OoIM sodium chloride at pH 8 containing a monoclonal antibody at 1 mg/ml, treated with a. 30 fold molar excess of a 10% w/v solution of 6 in N,N]dimethylformamide, for 24 hours. . .

-9]
Conditions for the coupling (b) (ii) between the compounds of f ormula. 6 and antibodies T-CHO are: aqueous 0.1M sodium acetate and aqueous 0*1M sodium chloride at pH 6 containing a monoclonal antibody at 1 mg/ml, treated with a 30 f old molar excess of a 5% w/v solution of 8 in the same buffer,. . .

doxorubicin (3f: RI=OCH3, R2=R4=OH, Rs=Hl, R3=NH2)
41 -epidoxorubicin (3g: %.=OCH3, R2=Rs=OH, R4=Hj R3=NH2) . all disclosed in previous patents see: F*Arcamone IIDOXORUBICINII Medicinal Chemistry. . .

. . .

groups by using
acetic anhydride and pyridine and subsequently deblocked at C]14 hydroxyl. position by means of aqueous hydrochloric acid to afford 6,11,41]tri=O]acetyl]N]trifluoroacetyl doxorubicin derivatives of formula 9 which are condensed with a compound of formula 8 in the same conditions as above re rted., to
PO
obtain,. . .

It is well known that in malignant tumors there is a high rate of glycolysis compared to normal tissue. This causes an increase in the production of lactate and thus a decrease of the pH in the tumor [see: H.M,Rauen et al.,, Z,Naturforsch, Teil B, 23 (1968) 1461). Also compounds of formula 4 41 6 and 7 may release the cytotoxic anthracycline within tumor tissues.

. . .

a two level specificity of
action of the compounds, the first one consisting in a preferential localization of the conjugate in the tumor

tissue by means of antigenic recognition, and the second one consisting in a preferential release of the drug in its active form in the tumor tissue by means of preferential acidic cleavage.

. . .

formation is assessed by chromatographic gel filtration procedures (Yu,D.S. et al., J.Urol. 1404, 415,t 1988) with simultaneous and independent detection of anthracycline and antibody at different wavelenghts, and by gel electrophoretic methods.

. . .

(Smith,P.K. et al.,
]18-
Anal.Biochem. 15O.- 76, 1985) or the Bradford dye assay (Bradford,M.M., Anal.Biochemo 72, 248p 1976)e
The antigen binding activity retention of the antibodies, after the conjugation procedures, is assessed by an ELISA method (Yu,,D.S. et al.,, i.Urol. 140, 415, 1988) and by cytofluorimetric methods (Gallego,,J. et ale, Int*J,Cancer 33p 737r 1984)o
The evalutation of the retention of cytotoxicity of conjugates in comparison with the parent drug is assessed by a test of. . . uptake of IH-Thymidine by the target cells, after an incubation time long enough to explicate the maximum cytotoxic effect (Dillmann.R.O. et al., Cancer Res.

antigen negative cell lines, after a short incubation time (Dillmann,R.O. et al., Cancer Res. 48, 6096, 1988)o
The acid sensitivity of the conjugate is evaluated by the above mentioned chromatographic methods after incubation of the compounds. . .

Alternatively, radiolabelling of the conjugates in the antibody moiety (12II) and/or in the anthracycline moiety (C) and HPLp analytical methods are employed for the evalutation of stability in plasma.

. . .

the compounds and the improvement of their therapeutic efficacy in comparison with the parent drug, are assessed in animal models of human trasplanted tumors. Nude mice bearing xenografts of human tumors are treated with suitable doses of conjugates, of free drug, of antibody, and of a physical mixture of drug and antibody, at equivalent doses, and the tumor growth is recorded and compared in the different treatment groups.

. . .

human melanoma cell line of an anti-
3
transferrin receptor immunoconjugate of the invention I.:, (line X-]-X-]-X), and of the parent anti-human transferrin receptor antibody OKT9 (line In the graph optical absorbance at 495 nm (y axis) is plotted against antibody concentration in ng/ml.

. . .

results of an evaluation, reported in Example 32 which follows, of the inhibition of cytotoxicity of the antitransferrin receptor conjugate 13 by unconjugated antibody. Line X]]-X --- X immunoconjugate 13 + OKT9 antibody and line 0 --]O --- immunoconjugate 13. The (3H) Thymidine incorporation, as a
Example 1 1
Preparation of Ethyl 2w(3,4mdihYdro=2H=PYTanm2myl) Methyloxyacetate (8B)

/0
2n \6CH]CHaOCHaCOOCaRs
3H11 I
C. . .

2e42 (COCH3)0 2e4m2*6 (CH2swNmCH2)j 2*82 MmOa]Oa]*CO),
3*5]3*7 (CH2]0a&CH2)r CH (4]CH30)1 5*13 W=H), 5.28 O]R,
61']H)o 5*52 (11]H)e
ExamPle-5
Preparation of 6,11,41]tri-O]acetyl]N]trifluoroacetylso
doxorubicin (9f)
N-trifjuoroacetyl]doxorubicin (3f: R3=NHCOCF2) (6*4 go
-
10 ffmle) was suspended in anhydrous methylene chloride
(750 ml) and added with triethylorthoformate (150 ml) and
p]toluensulfonic acid. . .

- 27 -
Example 6
Preparation of 14]O](2wcarboxytetrahydropyra=6]yl)doxorubicin,
X and Y isomers (211R.61IR and 211S,611S)e
4'f(A): Al](*Vf (R3=NH3); W = 0
2 to N*O C
OOOW IH]Ww
CH2 CH=
Nk]'- 00e
CH2
6,11,41]tri]omacetyl]N]trifluoroacetyldoxorubicin (10f) (5g,
6.5 mmole),. . .

acetone (95:5 by volume) to obtain the N]trifluoroacetyl
14]o](2-carbonyltrabydropyran]6]yl)doxorubicin (10f) (4e2 g,
yield 82%). Rt=0.41 (system D); FD]MS m/e 795 (M+)
IHN'MR (200M, CDC13) inter alia 6.

4f(A)(MM): X]0=3'f (R3=MM)fo W 0
40e
40CM]Co]
I
CH2
14]O](2.m:carboxytetrahydropyran]6]yl)doxorubicin (41f(A),
1*3 gg 2 mmole) . prepared as described in Example 6, was
dissolved in water (300 ml) and added with I]methoxy]2,21]
oxydiacetaldehyde. . .

R,=0.20 (system A); FD]MS m/e 868 (M+)*
- 30 -
Example 9
Preparation of 14]O](2mcarboxymethylaxymethyl]tetrahydropyran]
6]yl)doxorubicin: mixture of isomers 2R,6R; 2S,6S;
2.S,6R; 2R,6S.

4'f(B A']31 f (R3=NH2); W 0
209 R]CN20CH2=]
:;13 49]9]
CH2 CH2
CH2
6,11,41]tri]o]acetyl]N]trifluoroacetyl]doxorubicin (9ft 0*65g,
0.85 mmol), prepared as described in Example 5, was dissolved
in anhydrous methylene chloride, (50 ml) and treated with
compound 8B. . .

Exam2le 10
Preparation of .14]o](2]carboxymethylaxymethyl]tetrahydrom

pyranm6myl)]3']deamino]31(4]mor2holino)doxorubicin: mixture of isomers 211R,611R; 211SIVIS; 2S,6R; 2R,6S.

R,=0.48 (system B)
FAB-MS: 757 (M+)
- 32 -
Exam2le 11
Preparation of N]oxysucqinimidyl derivative of 14]O][2]earboxymetbyloxymethyl]tetrahydropyran]6]yll]3l] deamino]31(4]morpholino)doxorubicin: mixture of isomers 2RfSR; 2S,6S; 2S,6R; 2'R,6'S.

FAB]MS: 852 (13, MM+); 200 (100)
Example 12
Preparation of 14]o](2]carboxymethylox-ymethyl]tetrahYdro] P.Y.ran]6myl)]lt]deamino]3't2iS)]methoxy]4]mor2holinoI doxorubicin: mixture of isomers 2R,6R; 2S,6S; 2S,6R; 2RO, 6S a
4f(B)(MM): A]O--Vf (RI=MM); W 0
.2CH CH]CH20CH2CO]
000* I
62 CH2
'0]
CH2
]O](2]carboxymethyloxymethylmtetrahydropyran]6]yl) doxorubicin Wf(B), 0*3 g), prepared as described in Example 9, was converted into the title compound 4f (B) (MM) by treatment with l]methoxy]2,21]oxydiacetaldehyde. . .

Rt=0.50 (system B)
FABmMS: 787
Example 13
Preparation of N]oxysuqcinimidyl derivative of 14]o][2]carboxymethyloxymethyl]tetrahydropyran]6]yll]3t] deamino]31[2(S)]methoxy]4]morpholino)doxorubicin: mixture of isomers 211R,611R; 2S,6S; 2S,6R; 2R,6S.

7f(A)(MM): A]0=3'f (R3=MM)l W
21 6*1
;;]CH CHmCO-
&2
'0,] 000010CH2
CH2
- 34 -
K]oxysuccinimidyl derivative of 14]0](2]carboxytetrahydro] pyran]6]yl)w3l]deamino]31[2(S)]methoxy]4]morpholinyl]doxo] rubicin 5f (A) (MIQ e 100 mg I. prepared as described in Example S. was dissolved in anhydrous tetrahydrofurane (20 ml),, at OOC. . .
. . .
5f(A)(M (Example 8), in dimethylformamide (38 mcl) was added to I ml of a 2 mg/ml solution of purified mouse monoclonal anti]human melanoma antibody Epl (P.Giacomini, O.Segatto, P.G.Natali, Int.i.cancer 39, 1987, 729) in PBS pH 7.5 buffer. The
- 36 -
reaction mixture was stirred overnight at room temperature and clarified by. . . spectrophotometrically at 480 nm. The protein content was assayed with a colorimetric protein analysis (BCA, Pierce). The conjugate I contained 0.98 mg/ml of antibody with an anthracycline:protein ratio of 8.7:1. The chemicophysical profile of the product was determined by HPLC gel filtration analysis with dual wavelenght. . .

A]0=3'f (R3=MM); W 0 Z =]NH]N=CH]
2 46 CH OoICH_Co]
I
a CH2
400e
CH2
A solution of B72.3 antibody (US Pat.4,522,918, 1985) at 2*6 mg/ml in 0.1M phosphate buffer, pH 6, (1 ml) was treated with 0.1 ml of a 0.1. . . pH 7 The proteinowcontaining peak was collected (2 ml) and characterized as for Example 16, The conjugate 12 contained 0.45 mg/ml of antibody with an anthracycline/protein ratio of 1.3/1 and displayed an analytical profile analogous to the conjugate obtained in Example 16.

. . .
in K,,N=dimethylformamide (37 mcl) was added to I ml of a 2 mg/ml solution of a purified mouse monoclonal anti]human transferrin receptor antibody OKT9 (Sutherland,, R., Delia, D., Schneider, C., Newman, Rev Kemshead, J,,Greaves, Mo. Proc. Nat. Acad. Sci. USA, 78 (1981), 4515; ATCC CRL 8021). . .

. . .
(spectrophotometrically
- 38 -
at 480 rim) and for protein content (BCA Protein Assay Reagent, Pierce, Cat. 23225). The Conjugate contained 1.94 mg/ml of antibody and 80.9 mcg/ml of anthracycline, with an anthracycline : protein molar ratio of 9*5& The analytical profile of the product was evaluated. . .

. . .
9 e 1 , was obtained with operating as in Example 18 and using in place of OKT9, a solution of B72.3 antibody (2.3 mg/ml) [Schlom, J. et ale, US Pat. 4 522 918 (1985H and 43 mcl of the solution of 5f(A)(MM), Example 20
Preparation. . . with operating as in Example 18 and
- 39 -
using in place of OKT9,. a solution of an anti]epidermal growth factor receptor antibody (1.15 mg/ml) (BioMacor Cat.

. . .
of 3.9, was obtained with operating as in Example 18 and using in place of OKT9 a solution of the anti]transferrin receptor antibody B3/25 (0.5 mg/ml) (Boehringer Cat. No.

. . .
an anthracycline
protein ratio of 4.5 was obtained with operating as in Example IS and using in place of OKT9,, the antivewcolon carcinoma antibody B72.3 and 43 mcl of a 10E]2 M solution of 5f(B)(MG) (Example 11).

. . .
= 10le0 Z =]NH]
402CH :]]M]CH20CH2CO]
00
I I
C CH2
CH2
operating as in Example 18 and using in place of OKT9. the anti]colon carcinoma antibody B72.3 and 43 mcl of a lOE-2M solution of compound 5f(B)(MO), was prepared the title compound 13 containing 0.55 mg/ml of protein. . . 0 CHa]CHa
2 0#00 *S*NI ***<
CH H]Co] Z = N% 0000, N]Co]
62 00000' CE2 CH2]CKZ
**#%Csa

PolymL]Glutamic acid, M,. 2000]15000 (Sigma) (0.1 9) and 14]O](6mpiperazinecarbonyltetrahydropyran]2]yl)]3']deamino] 3'[2(S)]methoxy]4]morpholiny]doxorubicin [7f(A)(MMI, 0.03 g], (described in Example 14), wire dissolved in anhydrous dimethylformamide (2 ml) and stirred for three hours. After that, N]ethoxymcarbonyl]2methaxy]l ,. . .

By spectroscopic evaluation, the conjugate contains 12% (w/w %) of 31]deamino]31[2(S)]methoxy]4]morpholinyl]doxorubicin hydrochloride.

Example 30
Evaluation of the cell binding activity of the anti]trans] ferrin receptor conjugate 13 on a human melanoma cell line
Ref.: Matsui,. . . were
washed with PBS, a% FCS and incubated with different
- 45 -
concentrations of the conjugate 11 (Example 18), or of the OKT9 antibody, in PBS, 3% FCS# 100 melt 5w1OOO ng/ml for 1 h at 37OCe After three washings with PBS, 3%FCSt 100 mcl. . . nm with a BioRad EIA Reader Model 2550, The conjugate displayed a good retention of cell binding activity in comparison with the parent antibody, as shown in Fig* 1*
Example 31
Evaluation of the selective cytotoxicity of the antim transferrin receptor conjugate 13.

Ref.: Dillman, R. 0. et al., Cancer Res., 48 (1988) 6097
Ahmad, A. et al., Anticancer Res., 10 (1990) 837
MIG human melanoma cells (ATCC CRL 8021) were plated. . .
. . .
Results from a typical experiment are shown in Fig* 2*
Example 32
Inhibition of cytotoxicity of the antiamtransferrin receptor immunoconjugate 11 by unconjugated antibody
Ref.: Chaundhary, V. K. et al., Nature 339 (1989) 394
Batra, J. Ka et ale: Mole and Cell, Biol. 11 (1991)
2200
Siegal, C.. . . the
culture medium was removed and 50 mcl of fresh medium were added. Then,, 50 mcl of a solution of OKT 9 antibody in complete culture medium were added to triplicate wells (final concentration in wells from 7000 to 56 mcq/ml).
. . .
h at 46C, then 50 mcl of
different concentrations of the conjugate 13,, in complete culture medium, were added (final concentration of antibody in wells from 70 to 0.56 mcg/ml, corresponding to a 1:100
- 47 -
molar excess of free antibody added in the previous step).
. . .
continued at 370C for 7h. Cells were
harvested and radioactivity incorporation was evaluated as in Example 31,
As shown in Figure 3. free antibody addition inhibits the cytotoxicity of the immunoconjugate ever a range of doses of anthracycline content, thus confirming the receptor mediated effect of the. . .

Example 33
Doseearesponse inhibition of cytotoxicity of the anti-atransferrin receptor immunoconjugate 11 by unconjugated antibody
Ref.: Chaundhary, V. Ka et al.0 Nature 339 (1989) 394

Batra, J. Ka et al.: Mol. and Cell. Biol. 11 (1991)
2200
Siegal, C.. . . After that, the culture medium was removed and
mcl of fresh medium were added. Then, 50 mci of a
solution of OKT9 antibodv in complete culture medium were
added to triplicate wells (final concentration in wells from
2120 to 21.2 mcg/ml). Control wells were treated. . . were incubated
for Ih at
46C. Then, 50 mcl of the conjugate 13 in complete culture
medium, were added (final concentration of antibody in wells
2*12 mcg/ml). Cells were incubated, harvested and
radioactivity incorporation evaluated as in Example 31.

. . .
shows, a dosemresponse related inhibition of
- 48 -
conjugate cytotoxicity is effected by the pre]treatement of
cells with different excesses of unconjugated antibody,
confirming the receptor]mediated cytotoxicity of the
compounds.

As Table 1 indicates, specific conjugates are consistently
more efficient in inhibiting the growth of tumor cells in
comparison with control, non binding, conjugates. The ratio
between the ICSO may be taken as an index of the selectivity
of. . .

Compound 1 was evaluated in vivo against P388 murine
Leukemias resistant to Doxorubicin in comparison with
31]deamino]3112(S)]methoxym4]morpholinoldoxorubicin (DKK)
and doxorubicine Data are reported in Table 2.

P38S/DXIII
compound dose' T/C(2) TOX
(mg/kg)
1L4 5*2,(4) 200 0/10
Doxorubicin 1669 100 0/10
r)MM C S 0 a 09 192 0/39-
I 101 cells/mouse (P388/DX, Johnson) transplanted i,v. in
CDFI mice. Treatment on day 1 after inoculation of
tumor.

CLMEN. . .]CH2]1]C3H.] or]CH,,],
7a A conjugate according to any one of the
preceding claims, wherein the carrier is selected from a
polyclonal antibody, or fragment thereof comprising an
S antigen binding site, capable of binding to a tumor
associated antigen; a monoclonal antibody, or fragment
thereof comprising an antigen binding site, capable of
binding to an antigen preferentially or selectively
expressed on tumor cell populations; a peptide or protein
10 capable of preferentially or selectively binding to a tumor
cell; and a polymeric carrier.
A conjugate according to claim 7 , wherein the
carrier is selected from an anti]T]cell antibody, an
anti-CDS antibody, an anti]transferrin receptor
antibody,
15 and anti-melanoma antibody, an anti]carcinoma marker
antibody, an anti]ovarian carcinoma antibody, an
anti]breast
carcinoma antibody and an anti]bladder carcinoma
antibody.
9e A conjugate according to claim 7, wherein the
carrier is agrowth factor.
. . .

process as
claimed in claim 10.
12a A derivative of formula 4 as defined in claim
10,
A derivative according to claim 12, selected
from:
41]epi]4t]O-(2]carboxytetrahydropyran-6]yl)]3*]deamino]3t]
(4-morpholino)-daunorubicin;
14 (2]carboxytetrahydropyran]6]yl)]3']deamino-3'](2-
methoxy]4]morpholino)]doxorubicin; and
14]o](2]carboxymethyloxymethyl]tetrahydropyran]6]yl)]3']
deamino]3'](4-morpholino)]doxorubicin,
1 14 e A procesk' for the preparation of a derivative
of formula 4 as claimed in claim 12, which process comprises
ia). . . formula 41.
15a A derivative of formula 40 as defined in claim
4e
1 6a A derivative according to claim 15, selected
from:
41]epi O](2]carboxytetrahydropyran-6]yl)]daunorubicin;
14 (2-carboxytetrahydropyran]6]yl)]doxorubicin; and
14 (2-carboxymethyloxymethyl-tetrahydropyran yl)
25 doxorubicin.
- 58 -
1 7e A process for the preparation of a derivative
of formula 40 as claimed in claim 15, which process
comprises:
(a). . .

19 A derivative according to claim 18, selected
15 from:
4']ePi]4,]o](2]succinimidocarbonyl]tetrahydropyran]6]yl)]
3t]deamino]3g](4]morpholino)]daunorubicin;
14]O](2]succinimidocarbonyl]tetrahydropyran]6]yl)]3']
deamino-3f](2-methoxy morpholino)]doxorubicin; and
20 14]O](2]succinimidocarbonylmethyloxymethyl]tetrahydropyran]
6]YI)]3t]deamino-3f](4]morpholino)]doxorubicin.

=> d kwic 1

L7 ANSWER 1 OF 14 PCTFULL COPYRIGHT 2006 Univentio on STN
ABEN . . . polymer such as poly-glutamic acid, poly-aspartic acid or
poly-lysine. Also disclosed are methods of using the compositions for
treatment of tumors,
auto-immune disorders such as rheumatoid arthritis. Other embodiments
include the coating of
implantable stents for prevention of restenosis.

DETD . . .
FIELD OF THE INVENTION
The present invention relates generally to the fields of pharmaceutical
compositions to be used in the treatment of cancer, autoimmune
diseases and
restenosis. The present invention also relates to the field of
pharmaceutical
preparations of anticancer agents such as paclitaxel (Taxol. . .
. . .
INVENTION
Paclitaxel, an anti-microtubule agent extracted from the needles and
bark of
the Pacific yew tree, Taxus brevifolia, has shown a remarkable anti-
neoplastic effect
in human cancer in Phase I studies and early Phase II and III

trials (Horwitz et aL,
1993). This has been reported primarily in advanced ovarian and breast cancer.

Significant activity has been documented in small-cell and non-small cell lung cancer,
head and neck cancers, and in metastatic melanoma. However, a major difficulty in
the development of paclitaxel for clinical trial use has been its insolubility. . .
. . .
precursor extracted from the needles of Taxus baccata and esterified with a chemically synthesized side chain (Cortes and Pazdur, 1995). Various cancer
cell lines, including breast, lung, ovarian, and colorectal cancers and melanomas have
been shown to be responsive to docetaxel. In clinical trials, docetaxel has been used
to achieve complete or partial responses in breast, ovarian, head and neck cancers, and
malignant melanoma.
. . .
alcohol (50% v/v) and must be further diluted before administration (Goldspiel, 1994). Paclitaxel (Taxol TM) has shown significant activity in human
cancers, including breast, ovarian, non-small cell lung, and head and neck cancers
(Rowinsky and Donehower, 1995). It has also shown significant activity in patients
with advanced breast cancer who had been treated with multiple chemotherapeutic
agents (Foa et aL, 1994). As with most chemotherapeutic agents, however, the
maximum tolerated dose. . .
. . .
to be expensive. A
course of treatment may cost several thousand dollars, for examhle. There is the
added disadvantage that not all tumors respond to paclitaxel therapy, and this may be
due to the paclitaxel not getting into the tumor. There is an immediate need,
therefore, for effective formulations of paclitaxel and related drugs that are water
soluble with long serum half lives for treatment of tumors, autoimmune diseases such
as rheumatoid arthritis, as well as for the prevention of restenosis of vessels subject to
traumas such as angioplasty. . .
. . .
of taxane that has pharmaceutical properties different from that of paclitaxel. These compositions are shown herein to be surprisingly effective as anti-
tumor agents against exemplary tumor models, and are expected to be at least as
effective as paclitaxel, docetaxel, or other taxoid against any of the diseases. . . drawbacks associated
with the insolubility of the drugs themselves, and also provide the advantages of
improved efficacy and controlled release so that tumors are shown herein to be
eradicated in animal models after a single intravenous administration, as well as
providing a novel taxane. Poly-(I-glutamic. . . paclitaxel is shown

in the
examples hereinbelow to have a novel drug activity, in addition to having improved
the delivery to the tumor and providing a controlled release.

. . .
used to make water soluble
polymer conjugates of other therapeutic agents, contrast agents and drugs, including
paclitaxel, tarnoxifen, Taxotere, etopside, teniposide, fludarablne, doxorubicin,
daunomycin, emodin, 5-fluorouracil, FUDR, estradiol, camptothecin, retinoids,
verapamil, epothilones cyclosporin, and other taxoids. In particular, those agents with
a free hydroxyl group would. . .

. . .
also understood that the water soluble conjugates of the present invention
may be administered in conjunction with other drugs, including other anti-tumor or
anti-cancer drugs. Such combinations are known in the art. The water soluble
paclitaxel, docetaxel, or other taxoid, or in preferred embodiments the.
. . (PG-TXL), of the present invention may, in
certain types of treatment, be combined with a platinum drug, an antitumor agent such
as doxorubicin or daunorubicin, for example, or other drugs that are used in
combination with TaxolTm or combined with external or internal irradiation,. . .

. . .
tissue from irrelevant drug-
mediated toxicity (Maeda and Matsumura, 1989; Reynolds, 1995). On the other hand,
it is well established that malignant tumors often have disordered capillary
endothelium and greater permeability than non-nal tissue vasculature (Maeda and
Matsumura, 1989; Fidler et al., 1987). Tumors often lack a lymphatic vasculature to
remove large molecules that leak into the tumor tissue (Maeda and Matsumura, 1989).

Thus, a polymer-drug conjugate that would normally remain in the vasculature may
selectively leak from blood vessels into tumors, resulting in tumor accumulation of
active therapeutic drug. The water soluble polymers, such as, in preferred
embodiments PG-TXL, may have pharmacological properties different from non-
conjugated drugs (ie. paclitaxel). Additionally, polymer-drug conjugates may act as
drug depots for sustained release, producing prolonged drug exposure to tumor cells.

. . .
to solubilize otherwise insoluble compounds. At present, a
variety of synthetic and natural polymers have been examined for their ability to
enhance tumor-specific drug delivery (Kopeeek, 1990, Maeda and Matsumura, 1989).

. . .
However, only a few are known by the present inventors to be currently undergoing

clinical evaluation, including SMANCS in Japan and HPMA-Dox in the United
Kingdom (Maeda, 1991; Kopecek and Kopeckova, 1993).

. . .

oxo-7,1 1-methano-IH-cyclodeca[3,4]benz-[1,2-b]oxet yI ester. It is understood that paclitaxel and docetaxel are each more effective than the other against
certain types of tumors, and that in the practice of the present invention, those tumors
that are more susceptible to a particular taxoid would be treated with that water
soluble taxoid or taxane conjugate.

. . .

etoposide,
teniposide, fludarabine, verapamil, or cyclosporin, for example, or even to water
soluble agents such as 5 fluorouracil (5 FU) or fluorodeoxyuridine (FUDR),
doxorubicin or daunomycin.

. . .

to an animal or human subject. Therefore,
the present invention may also be described as a pharmaceutical composition
comprising a chemotherapeutic or anti-cancer drug such as paclitaxel, docetaxel, or
other taxoid conjugated to a high molecular weight water soluble polymer or to a
chelator. The. . . acids, poly-aspartic acids, poly-lysine, or a chelator, preferably DTPA. It is
also understood that a radionuclide may be used as an anti-tumor agent, or drug, and
that the present pharmaceutical composition may include a therapeutic amount of a
chelated radioactive isotope.

. . .

may be described as a method
of determining the uptake of a chemotherapeutic drug such as paclitaxel, docetaxel, or
other taxoid by tumor tissue. This method may comprise obtaining a conjugate of the
drug and a metal chelator with a chelated metal ion, contacting tumor tissue with the
composition and detecting the presence of the chelated metal ion in the tumor tissue.

The presence of the chelated metal ion in the tumor tissue is indicative of uptake by
the tumor tissue. The chelated metal ion may be a radionuclide and the detection may
I 0
be scintigraphic. The tumor tissue may also be contained in an animal or a human
subject and the composition would then be administered to the. . .

The present invention may also be described in certain embodiments as a method of treating cancer in a subject. This method includes obtaining a composition
comprising a chemotherapeutic drug such as paclitaxel, docetaxel. or other taxoid
conjugated to. . . chelator and dispersed in a pharmaceutically acceptable solution and administering the solution to the subject in an amount
effective to treat the tumor. Preferred compositions comprise paclitaxel, docetaxel, or

other taxoid conjugated to a water soluble polyamino acids, including but not limited to poly (I-aspartic. . . acid), poly (d-glutamic acid), or poly (dl-glutamic acid). The compositions of the invention are understood to be effective against any type of cancer for which the unconjugated taxoid is shown to be effective and would include, but not be limited to breast cancer, ovarian cancer, malignant melanoma, lung cancer, head and neck cancer. The compositions of the invention may also be used against gastric cancer, prostate cancer, colon cancer, leukemia, or Kaposi's Sarcoma. As used herein the term treating cancer is understood as meaning any medical management of a subject having a turaor. The term would encompass any inhibition of tumor growth or metastasis, or any attempt to inhibit, slow or abrogate tumor growth or metastasis. The method includes killing a cancer cell by non-apoptotic as well as apoptotic mechanisms of cell death. The method of treating a tumor may include some prediction of the paclitaxel or docetaxel uptake in the tumor prior to administering a therapeutic amount of the drug, by methods that include but are not limited to bolus injection or infusion,. . .

. . .

include any of the imaging techniques discussed above in which a paclitaxel-chelator-chelated metal is administered to a subject and detected in a tumor. This step provides a cost effective way of determining that a particular tumor would not be expected to respond to DTPA-paclitaxel therapy in those cases where the drug does not get into the tumor. It is contemplated that if an imaging technique can be used to predict the response to paclitaxel and to identify patients. . .

The assumption is that if there is no reasonable amount of chemotherapeutic agent deposited in the tumor, the probability of tumor response to that agent is relatively small.

. . .

cases to paclitaxel when administered in the standard Cremophor formulation (US Patent 5,583,153, incorporated herein by reference). As in the treatment of tumors, it is contemplated that the effectiveness of the water soluble taxoids or taxane of the present invention will not be diminished by. . .

. . .

invention may be used in combination with other drugs, suc as an angiogenesis inhibitor (AGM-1470) (Oliver et aL, 1994), or other anti-cancer drugs, such as methotrexate.

In certain aspects of the invention, the amount of anti-tumor drug conjugated

per water soluble polymer can vary. At the lower end, such a composition may
comprise from about 1%, about 2%,. . .

Preferred anti-tumor drugs include paclitaxel, docetaxel, or other taxoids, and
preferred water soluble polymers include water soluble amino acid polymers.

In certain other aspects of the invention, the number of molecules of anti-
tumor drug conjugated per molecule of water soluble polymer can vary. At the lower
end, such a composition may comprise from about. . .

Preferred anti-tumor drugs include paclitaxel, docetaxel, or other taxoids, and
preferred water soluble polymers include water soluble amino acid polymers. The
preferred number of anti-tumor drug molecules conjugated per molecule of water
soluble polymer is about 7 molecules of antitumor drug per molecule of water soluble
polymer.
. . .
other aspects. of the invention, biological
1 5 functional equivalents of water soluble amino acid-taxoid polymers may be further
identified by improved anti-tumor cell activity, relative to the anti-tumor cell activity
of the unconjugated water soluble amino acid polymer used to produce the particular
water soluble amino acid polymer-taxoid composition by. . .

FIG. 3. Antitumor effect of DTPA-paclitaxel on MCa-4 mammary tumors.

FIG. 4. Median time (days) to reach tumor diameter of 12 mm after treatment with
I 0 paclitaxel, DTPA-paclitaxel and PEG-paclitaxel.

FIG. 5. Gamma-scintigraphs of mice bearing MCa-4 tumors following intravenous
injection of 1 1 1 In-DTPA-paclitaxel and III In-DTPA. Arrow indicates the tumor.

FIG. 7A. Anti-tumor effect of PG-TXL against syngeneic OCA-I ovarian carcinoma
tumor in female C3Hf/Kam mice. Drugs were injected intraveneously in a single
dose. Data are presented as mean ± standard deviation of tumor volumes. a, Mice
bearing OCA-1 tumor were injected with -El-, PG control (800 mg/kg; n--9);
paclitaxel (80 mg/kg; n=7); -A-, paclitaxel (80 mg/kg) plus PG (800 mg/kg;. . .

FIG. 7B. Anti-tumor effect of PG-TXL against 13762F tumor in female rats.
represents PG control (220 mg/kg; n=7), -,&- represents paclitaxel (20 mg/kg; n=5), -
A- represents paclitaxel (40 mg/kg;. . .

FIG. 7C. The antitumor effect of PG-TXL on mice bearing MCa-4 mammary

carcinoma tumors. represents the response to a single i.v. dose of saline, -A- represents the response to a single i.v. dose of PG. . .

FIG. 7D. The antitumor effect of PG-TXL against soft-tissue sarcoma tumor (FSa-11) in mice. represents the response to a single Lv. dose of saline, represents the response to a single i.v. dose of. . .

FIG. 7E. The antitumor effect of PG-TYL against syngeneic hepatocarcinoma tumor (HCa-I) in mice. represents the response to a single i.v. dose of saline, -A- represents the response to a single i.v. dose. . .

FIG. 9. Antitumor effect of PEG-paclitaxel on MCa-4 mammary tumors. represents the response a single Lv. injection with a saline solution of PEG (60 mg/ml), represents the response to the Cremophor/alcohol. . .

j
FIG. 12A. Time-dependent OCA-1 tumor content of radioactivity following injection of either PGTHIpaclitaxel and [3 H]paclitaxel into mice. Open bars represents PG-TXL radioactivity after injection of. . .

FIG. 12B. Conversion of PGTHIpaclitaxel to CH]paclitaxel within OCA-I tumor.

FIG. 13. Kinetics of apoptosis in OCA-1 tumors after a single i.v. dose of 160 mg equiv. paclitaxel/kg of PG-TYL (MTD) and 80 mg/kg paclitaxel (MTD). -[]- represents the response. . .

FIG. 14. Survival of nude mice with human ovarian cancer cells (SKOV3ipl) treated with PG-TXL. Five days after tumor injection, the mice were injected i.v. with the PG-paclitaxel (PG-TXL), or PG control. Injections of PG-TXL were administered every seven days (Y). . .

. . .
invention arises from the discovery of novel, water soluble formulations of paclitaxel and docetaxel, and the surprising efficacy of these formulations against tumor cells in vivo. Poly (1-glutamic acid) conjugated paclitaxel (PG-TXL) administered to mice bearing ovarian carcinoma (OCA-1) caused significant tumor growth delay as compared to the same dose of paclitaxel without PG. Mice treated with paclitaxel alone or with a combination of free paclitaxel and PG showed delayed tumor growth initially, but tumors regrew to levels comparable to an untreated control group after ten days. Moreover, at the maximum tolerated dose (MTD) of the PG-TXL conjugate, (160 mg equiv. paclitaxeUkg), the growth of tumors was completely suppressed, the tumors shrank, and mice observed for two months following treatment remained tumor free (MTD: defined as the

maximal dose that
produced 15% or less body weight loss within two wk after a single. .
. i.v. injection). In
a parallel study, the antitumor activity of PG-TXL in rats with rat
mammary
adenocarcinoma (13762F) was examined. Again, complete tumor
eradication at 40-
60 m equiv. paclitaxel/kg of PG-TXL was observed. These surprising
results
9
demonstrate that the polymer-drug conjugate, PG-TXL, successfully
eradicates well
established solid tumors in both mice and rats after a single
intravenous injection.

In addition to the remarkable antitumor (breast, ovarian, etc.) data in
syngeneic
mice, good activity of PG-TXL against human breast cancer
(MDA-435) and ovarian
cancer (SKOV3ipl) in nude mice has recently been observed.
Nude mice are special
animals with incomplete immune systems in which human tumors
can grow.

The data presented herein have led the present inventors to conclude
that PG-
TXL is a novel species of taxane that. . . PG-TXL appears to re-main
for a much longer
period. This is contemplated to offer a distinct advantage in that
prolonged exposure
of tumors to the drug may result in an enhanced response. The
rate of conversion of
PG-TXL to paclitaxel is slow, with less. . . paclitaxel within 48 h
after injection of the
paclitaxel-polyrner complex. This finding suggests that the novel drug,
PG-TXL, may
produce death within tumor cells in a manner which is not
simply due to the gradual
release of paclitaxel itself.

Further evidence of the novelty of PG-TXL is that relatively high levels
of
radioactivity from radiolabeled PG-TXL appear in tumor tissue
shortly after injection.

However, only small amounts of radioactivity within tumor
tissue are due to the
release of free paclitaxel. Furthermore, the percent of radioactivity
within tumor
tissue due to paclitaxel itself does not appreciably increase with time
suggesting again
that PG-TXL is a minimal prodrug for the gradual. . . of tissue
distribution
and again suggest that there are several mechanisms or ways in which
PG-TXL may
lead to the death of cancer cells which are different from
those for paclitaxel.

Recent analyses of tumor tissues from mice treated with
paclitaxel suggests
that, as expected, this drug results in the formation of many apoptotic
bodies within
the tumor itself. Apoptosis is a mechanism in which cells
commit self-induced death

or programmed cell death, a natural process used by an organism in wound healing
and tissue remodeling. Tumors from mice treated with PG-TXL had far fewer
apoptotic bodies compared to free paclitaxel but had an increased incidence of tumor
necrosis and edema suggesting that paclitaxel and PG-TXL may result in tumor cell
death by two distinctly different pathways.

These studies, and those described in the specific examples, demonstrate that
PG-TXL is a new taxane which is not only extremely active against breast and ovarian
cancers, and appears to have limited side affects. It is now clear that the polymer
conjugation of paclitaxel results in a compound. . .
. . .
invention is the inclusion of molecules in the polymeric composition that are effective to target the therapeutic composition to a
disease or tumor site or to a particular organ or tissue. Many of such targeting
molecules are known in the art and may be conjugated to the water soluble anti-tumor
compositions of the present invention. Examples of such molecules or agents would
include, but not be limited to antibodies such as anti-tumor antibodies; anti-cell
receptor antibodies; tissue specific antibodies; hormonal agents such as octreotide,
estradiol and tamoxifen; growth factors; cell surface receptor ligands; enzymes;
hypoxie agents such as misonidazole and erythronitroimidazole;. . .
. . .
melanoma cell line. DTPA-paclitaxel did not show any significant difference in
antitumor effect as compared to paclitaxel against an MCa-4 mammary tumor at a
dose of 40 mg/kg body weight in a single injection. Furthermore,]-] II Indium labeled
DTPA-paclitaxel was shown to accumulate in the MCa-4 tumor as demonstrated by
gamma-scintigraphy, demonstrating that the chelator conjugated anti-tumor drugs of
the present invention are useful and effective for tumor imaging.
. . .
present invention provide
significant advances over prior methods and compositions, as the water-soluble
paclitaxels are projected to improve the efficacy of paclitaxel-based anti-cancer
therapy, by providing water soluble and controlled release paclitaxel derived
compositions that also have different antitumor properties than unmodified paclitaxel.
. . .
for solvents that are associated with side effects
seen with prior paclitaxel compositions. In addition, radiolabeled paclitaxel, which is
shown to retain anti-tumor activity, will also be useful in the imaging of tumors.

Further, the present invention allows one to determine whether a paclitaxel will be taken up by a particular tumor by scintigraphy, single photon emission computer tomography (SPECT) or positron emission tomography (PET). This determination may then be used to predict the efficacy of an anti-cancer treatment. This information may be helpful in guiding the practitioner in the selection of patients to undergo chelator-paclitaxel therapy.

These salts will be useful as therapeutic agents for tumor treatment. Secondly, DTPA-paclitaxel or other paclitaxel-chelating agents will be useful as diagnostic agents which, when labeled with radionuclides such as III In or 99mTc, may be used as radiotracers to detect certain tumors in combination with nuclear imaging techniques.

. . .

acids, exists chiefly to orientate amino acid side chains in such a way as to facilitate molecular interactions, such as those of antibody and antigen. A peptide mimetic is thus designed to permit molecular interactions similar to the natural molecule.

. . .

and accelerated atherosclerosis is smooth muscle cell (SMC) proliferation (Phillips-Hughes and Kandarpa, 1996).. Since SMC phenotypic proliferation after arterial injury mimics that of neoplastic cells, it is possible that anti-cancer drugs may be useU to prevent neointimal SMC accumulation. Stents coated with polymer-linked anti-proliferative agents that are capable of releasing these agents. . .

. . .

the conjugation of paclitaxel to a water-soluble polymer, poly (1-glutamic acid) (PG) and the efficacy of the preparation against a variety of tumors in mice and rats. The potential of water-soluble polymers used as drug carriers is well established (Kopecek, 1990; Maeda and Matsumura,. . .

However, poly-aspartic acid may be conjugated to anti-tumor drugs using the reaction scheme described herein for PG-TXL.

In Vivo Antitumor Activity

All animal work was carried out at the animal facility at M.D. Anderson Cancer Center in accordance with institutional guidelines. OH/Karn mice were bred and maintained in a pathogen-free facility in the Department of Experimental Radiation. . .

The tumor growth delay induced by PG-TXL was measured in mammary ovarian carcinoma (OCA-1) implanted in C3Hf/Kam mice. All tumors

were
syngeneic to this strain. Solitary tumors were produced in the
muscle of the right
5
thigh of female C3H/Karn mice (25-30g) by injecting 5 x 10 murine
ovarian
carcinoma. . . or
fibrous sarcoma (FSa-11). In a parallel study, female Fischer 344 rats
(125-150 g)
were injected with 1.0 x 10 5viable 13762F tumor cells in 0.1
ml PBS. Treatments
were initiated when the tumors in mice had grown to 500 mm 3
(10 mm in diameter),
or when the tumors in rats had grown to 2400 mm 3 (mean
diameter 17 mm).
. . .
mg equivalent paclitaxel/ml), and
paclitaxel. was dissolved in Cremophor EL& vehicle (6 mg/ml). Data are
presented as
mean + standard deviation of tumor volumes. In control
studies, saline (0.6 ml),
Crernophor vehicle [50150 Cremophor/ethanol diluted with saline (1:4)],
PG solution
in saline, and paclitaxel plus. . .

Paclitaxel/kg body weight. Tumor growth was determined daily
(FIG. 7A, 713, 7C,
7D and 7E) by measuring three orthogonal tumor diameters.
Tumor volume was
calculated according to formula (A x B x Q/2. Absolute growth delay
(AGD) in
mice is defined as the time in days for tumors treated with
various drugs to grow from
500 to 2,000 mm3in mice minus the time in days for tumors
treated with saline
3 3
control to grow from 500 to 2,000 mm . When the tumor size
reaclTed 2000 MM, the
tumor growth delay was calculated; the mice were sacrificed
when tumors were
3 -TXL group were (n = 6 and 7), other each group
approximately 2500 mm . The PG
were (n = 5).. . . paclitaxel in rats in
comparison with paclitaxeUCremophor. Table 3 summarizes the data
concerning the
effect of PG-TYL against MCa-4, FSa-II and HCa-I tumors in
mice. The data are also
summarized in FIG. 7A-FIG. 7E.
. . .
1/4 16.7 6 15
Paclitaxel 5 40 0/3 17.9 6 16
Paclitaxelb 20 015 17.0 5 N/A
Drugs were administered intravenously into 13762F tumor
-bearing Fischer rats
(female, 130 g) in a single injection.

Table 3: The Antitumor Effect of PG-TXL Against
Different Types of In vivo Murine Tumors
r6wo]- AGff-
Tumor Drug' Time to G t -test'-
500 - 2000 mm 3
MCa-4 Saline 4.8±0.8 (5) - -
PG (0.6 g/kg) 9.3±1.1 (4) 4.5. . .

b Tumor growth was determined by daily measurement of three orthogonal diameters with calipers and the volume was calculated as (A x B. . .

c Absolute growth delay (AGD) defined as the time in days for tumors treated with various drugs to grow from 500 to 2000 mm 3 minus the time in days for 3 tumors treated with saline control to grow from 500 to 2000 mm.

PG-TXL is most effective against MCa-4 and OCA-I tumors. S(?cond, PG-TXL is more effective than paclitaxel on equivalent Ing paclitaxel basis in the case of MCa-4, HCa-I, and on OCA- I tumors, and is remarkably potent at its maximum tolerated dose (MTD).

. . . established rat mammary adenocarcinoma 13762F was examined. Female Fischer 344 rats (125-150 g) were injected with 1.0 x 10 5viable 13762F tumor cells in 0. I ml PBS. Once tumors reached a mean volume of 2000 mm 3 (mean diameter, 1.6 cm), animals were treated using a similar protocol as described above. Tumor growth was determined daily by measuring three orthogonal tumor diameters. Tumor volume was calculated according to the formula (AxBxC)/2. A single dose of PG-TXL in saline or paclitaxel in a Cremophor ELO. . . EL] vehicle [50150 Cremophor/ethanol diluted with saline (1:4)], PG solution in saline and paclitaxel plus PG were used.] Again, complete tumor eradication at the MTD of PG-TXL (60 mg equivalent paclitaxel/kg) was observed.

PG-TXL given at a lower dose of 40 mg equivalent paclitaxel/kg also resulted in complete tumor regression (FIG. 7B). In contrast, the MTD of paclitaxel in Cremophor EL] was less than 20 mg/kg. Paclitaxel at this dose caused a tumor growth delay (Tumor growth delay is defined as the time in days for tumors treated with the test drugs to grow from 2,000 mm 3to 10,000 mm3 minus the time in days for 3 3 tumors treated with saline control to grow from 2,000 mm to I 0,000 mm .) of only 5 days, whereas the same equivalent paclitaxel dose of PG-TYL resulted in a tumor growth delay of 23 days (FIG. 7B).

Studies of Nude Mice Injected with Human Breast Cancer and Treated with PG-TXL

Nude mice were injected with 2 x IO 6MDA Lung2 cells (a variant of the MDA-MB-435 human breast cancer cell line) into the mammary fatpad. When the tumors reached 5 mm mean diameter, (27 days after tumor injection), mice were treated with an i.v. injection of PG-TXL or the various controls

(see-Table 4). Tumor measurements were taken weekly. Tumors that reached 1.5 cm were removed surgically. All mice were killed at 120 days, and remaining tumors removed and weighed. Mice were examined for metastases, and lungs processed for histology, with single sections of the organs scored for the. . .

Table 4

Treatment	Tumor take'	Mean tumor Wt(g)b	No. tumors regressed'	Lung metastases d
PBS	5/6	1.3	0.24	4/5 (80%)
Cremophor	9/9	1.26	0.67	4/8 (50%)
PGA	10/10	1.13	0.7	4/7 (57%)
Taxol TM. . . mg/kg	10/10	1.23	0.38	2/10 5/8 (62.5%)
PG-TXL 120 mg/kg	9/10	0.925	0.12	4/8 1/4 (25%)

a Number of mice with 5mm tumors at time of therapy/number of mice injected

b Mean weight of tumors removed at time of autopsy

c Number of tumors that had regressed at time of autopsy

d Number of mice with lung metastases (either macroscopic or found in histology preparations)/number of mice with tumors. Some discrepancies between tumor take and number mice with tumors in this column due to sacrifice or deaths of animals for non-related reasons, e.g, developing Staphylococcus abscesses. One mouse in PG-TXL 120. . .

. . . single bolus of PG-conjugated paclitaxel (PG-TXL) was given, at a drug equivalent of 120 mg/kg paclitaxel, it is apparent that the MDA-435 cancer cell line responds to the drug and that this formulation of the drug is much better tolerated than when Cremophor is. . .

In the breast cancer study using MDA-MB-435, only the higher dose of PG-TXL inhibited the growth rate of the mammary fatpad tumors. From the growth curve I 0 it was apparent that tumor growth resumed approximately 30 days after the single dose of conjugate. However, the growth curve does not reveal that in the PG-TXL 120 mg/kg group there were a number of tumor regressions. As shown in Table 3, the incidence of lung metastasis in the mice with residual tumors was also reduced.

. . . the numbers of mice in the study are small, they do suggest that the therapy was effective in reducing both local tumor growth and incidence of metastasis.

. . . In this study design it is not possible to distinguish whethersa lower incidence of metastasis is due to a reduction of tumor mass of the primary site, or due to a direct effect on any micrometastases that may have already been established at.

. .

In Vivo Therapy of Human Breast Cancer Using Multiple Injections of PG-TXL

To test the effect of multiple injections of PG-TXL, nude mice were injected with 2 x 10 6; MDA Lung 2 cells (a variant of the MDA-MB-435 human breast cancer cell line) into the mammary fatpad. When the tumors reached 5 nu-n mean diameter, the treatments were started, and repeated at 14 day intervals (day 24, 38, 52) for a total of three injections. Tumor measurements were taken weekly. The mice were killed on day 105 after tumor cell injection, and the tumor weights and incidence of metastasis recorded. The lungs were processed for histology, and single sections scored for the presence of micrometastases. The. . .

Table 5

Treatment	Tumor take'	Mean weight(g) b	Tumors Metastasisd	regressed'
None	4/5	1.83 0.15	4/4 (I 00%)	
PG-control	6/10	1.7 0.11	5/6 (83%)	
PG-TXL/60 mg	7/10	1.36 0.28 -	6/7 (86%)	
PG-TXL/120 mg	8/10	0.97 ± 0.22 p = 0.01 le	2/8	2/6 (33%)

Legend: a Number of mice with 5 mm tumors at the time of therapy/number of mice injected
b Mean weight of tumors (±SEM)
c Number of tumors that had regressed at the time of autopsy
d Number of mice with lung metastases, either macroscopic or microscopic/number of mice with tumors
e p value from unpaired t test comparing tumor weight of treated mice with the control PG group.

In Vivo Therapy of Human Ovarian Cancer Using PG-TXL Conjugate

Nude mice were injected i.p. with the human ovarian cancer cell line, SKOV3ipl. Five days after tumor injection, the mice were injected ix. with the PG-paclitaxel (PG-TXL), at concentrations equivalent to 120 mg/kg or 160 mg/kg of paclitaxel.. . .

Table 6

Treatment	Tumor take a	Median survival (range) b	Ascite-i' Mean vol (ml) d
None	10/10	56 (38 - 98)	8/10 2.2 ± 1.6
PG-control. . .		2.2 ± 1.6	
PG-120	7/8	82 (59 - 98)	3/7 2.7 ± 1.4
PG-160	3/50	84 (34'. 98)	0/3

Legends: a Incidence of tumor/number of mice injected
b median survival time in days
c incidence of ascites/number of mice with tumor
d mean volume (and s.d.) of ascites
e these mice only received a single dose of PG-paclitaxel, 160 mg/kg, and does not include. . . the mice that dies within 5 days of the treatment

f. the mouse that was killed on day 34 had minimal tumor burden, but was paraplegic (possible toxicity?).

The PG-TXL 120 mg/kg significantly extended the survival of the mice with intraperitoneal SKOV3ipl, (a human ovarian cancer cell line which overexpresses HER21neu), compared with mice injected with PG alone. Multiple doses and/or increasing the dose of conjugate may significantly reduce the tumor incidence in addition to extending survival.

In the nude mice studies above, the growth curves show that although breast cancer growth is checked by paclitaxel, especially with the higher dose conjugated with PG. tumor size continues to increase about a month after the therapy. A second (or third) round of therapy may have caused the tumor growth to plateau, or give more tumor regressions. The growth curves do not include the tumors that regressed - as shown in Table 4, the tumors shrank/disappeared in 50% of the mice treated with the highest dose of PG-TXL, and of the 4 animals with progressively growing tumors at the end of the study, only one had micrometastases in the lungs. So the treatment that reduced growth of the primary tumors also reduced the incidence of metastasis. The incidence of metastasis in all other therapy groups, including the control groups of Cremophor and. . .

Antitumor Effect on Mammary Carcinoma (MCa-4) Tumor Model.

. . .

and 40 mg equivalent paclitaxel/kg body weight. In control studies, saline and absolute alcohol/Cremophor 50150 diluted with saline (1:4) were used. Tumor growth I 0 was determined daily, by measuring three orthogonal tumor diameters. When the tumor size reached 12 mm in diameter, the tumor growth delay was calculated. The mice were sacrificed when tumors were approximately 15 mm.

The tumor growth curve is shown in FIG. 3. Compared to controls, both 1 5 paclitaxel and DTPA-paclitaxel showed antitumor effect at a dose of 40 mg/kg. The data were also analyzed to determine the mean number of days for the tumor to reach 12 mm in diameter. Statistical analysis showed that DTPA-paclitaxel delayed tumor growth significantly compared to the saline treated control at a dose of 40 mg/kg ($p < 0.01$). The mean time for the tumor to reach 12 mm in diameter was 12.1 days for DTPA-paclitaxel compared to 9.4 days for paclitaxel (FIG. 4).

. . .

C3Hf/Kam mice were inoculated with mammary carcinoma (MCa-4) in the muscles of the right thigh (5 x IO 5cells). When the

tumors had grown to 12
mm in diameter, the mice were divided into two groups. In group I, the mice were
anesthetized by. . . from the plasma, rapid and high excretion in the urine with minimal
retention in the kidney and negligible retention in the tumor, the liver, the intestine
and other organs or body parts. In contrast, III In-DTPA-paclitaxel exhibited a
pharmacological profile resembling that of paclitaxel. . .
. . .
significant amount of 'In-DTPA-paclitaxel was also excreted through kidney, which on ly played a minor role in the clearance of paclitaxel. The
tumor had significant uptake of 'In-DTPA-paclitaxel. These results demonstrate
that ... In-DTPA-paclitaxel is able to detect certain tumors and to quantify the uptake
of III In-DTPA-paclitaxel in the tumors, which in turn, may assist in the selection of
patients for the paclitaxel treatment. In contrast, the smaller PG-TXL conjugate has a
different distrubution than DTPA-paclitaxel, and partly localizes in the liver and
tumors of test animals.

Antitumor Effect of PEG-Paclitaxel Against MCa-4 Tumor in Mice
To evaluate the antitumor efficacy of PEG-paclitaxel against solid breast
I 0 tumors, MCa-4 cells (5 x IO 5cells) were injected into the right thigh muscle of female
C31-1f/Kam mice. As described in Example I with the DTPA-paclitaxel, when the
tumors were grown to 8 mm (Approx. 2 wks), a single dose of paclitaxel or PEG-
paclitaxel was given at 10, 20. . .
. . .
absolute alcohol:Cremophor (1: 1) diluted with saline
(1:4) and PEG solution in saline (600 mg/kg body weight) were used in control
studies. Tumor growth was determined daily, by measuring three orthogonal tumor
diameters. When the tumor size reached 12 mm in diameter, the tumor growth delay
was calculated.

The tumor growth curve is shown in FIG. 9. At a dose of 40 mg/kg, both
PEG-paclitaxel and paclitaxel effectively delayed tumor growth. Paclitaxel was more
effective than PEG-paclitaxel, although the difference was not statistically significant.

Paclitaxel treated tumors required 9.4 days to reach 12 mm in diameter whereas PEG-
paclitaxel-treated tumors required 8.5 days. Statistically, these values were significant
(p > 0.05) as compared to their corresponding controls, which were 6.7 days. . .
. . .
PG-TXL and paclitaxel
pharmacological properties in their ability to promote in vitro assembly of tubulin, to
inhibit cell growth against rat mammary tumor cell line 13762F

tind human breast
tumor cell lines, to induce p53 protein, and to rescue a
paclitaxel-dependent mutant
cell line. Paclitaxel's release from PG-TXL in vivo was. . .

Effects of Poly-Glutamic Acid-Paclitaxel (PG-TXL) on the Growth of Rat
and
Human Tumor Cell Lines In Vitro
To evaluate whether the superior antitumor activity of PG-TXL observed
in
animals is due to increased cytotoxicity, PG-TXL and paclitaxel were
compared for
their ability to inhibit cell growth against the established rat mammary
tumor cell line
13762F. The effect of PG-TXL on cell growth was examined by &,plating
efficiency
assay. Rat 13762F cells were seeded (200. . .

In a similar study, the effect of PG-TXL on cell growth of human breast
cancer cell lines was examined by MTT assay after 3 days of
continuous exposure.

. . .
min). Slower clearance of PG-
TXL from the blood was a design feature of the polymer-drug conjugate
with the goal
of improving tumor uptake. Surprisingly, the rate of
conversion of PG-TXL to
paclitaxel in plasma is slow with less than 0.1% of the radioactivity.
. .

In a separate study, mice bearing OCA-1 tumors were prepared
as described
3
previously. When the tumor reached 500 mm . animals were
injected with a dose of
20 mg equivalent paclitaxel/kg of [3H]paclitaxel or PG-[3HJTXL. . .

Animals were killed at 2. 5] % 24] 48, and 144 h postinjection.
Tumors were
removed, weighed, and homogenized with 3 volume of PBS (w/v). Percent of
injected dose per gram tissue is calculated based on total radioactivity
associated with
the tumor, which was determined by counting prepared tissue
homogenate aliquots.

. . .
The eluting solvent (methanol:watter = 2: 1) was run at 1.0 ml/min. The
uptake of
1 5 total drugs in OCA-1 tumor was expressed as a percentage
of the administered dose
per gram of tissue and the association of radioactivity within OCA-1
tumor as free
paclitaxel was expressed as dpm per gram tissue.

Quantitative assessment of tumor uptake in C3Hf/Kam mice
showed that
relatively high levels of radioactivity from radiolabeled PG-TXL appear
in tumor
tissue shortly after injection (FIG. 12A) as compared to radiolabeled
paclitaxel.

However, only small amounts of radioactivity within tumor
tissue are due to the
release of free paclitaxel (FIG. 1213). Data are presented in FIG. 12A
and FIG 12B as

mean+-SD from 3 mice per time point. The percent of radioactivity within tumor
tissue due to paclitaxel does not appreciably increase with time suggesting that PG-
TXL is not simply a prodrug for the gradual. . .
. . .
procedures. These
polyamino acid-paclitaxel conjugates had similar paclitaxel content, aqueous
solubility, and molecular weight (30-40K). In C3Hf/Kam mice bearing murine OCA-
I ovarian cancer (500 mm 3 at time of treatment), a single i.v. injection of
poly(I-glutamic acid)-paclitaxel at 80 mg equiv. paclitaxel/kg body weight produced a
tumor growth delay of 21 days vs. saline treated controls. Poly(d-glutamic
acid)-paclitaxel was as effective as poly(I-glutamic acid)-paclitaxel. However,
paclitaxel conjugated with poly(I-aspartic acid) was completely inactive against OCA-
1 tumor. In a separate study, the antitumor activity of polymer-paclitaxel conjugates
of different molecular weight (1K, 13K, and 36K) was compared. Conjugates. . .

To assess the mechanism of PG-TXL associated antitumor activity, histological sections of OCA-1 tumors excised from paclitaxel and PG-TXL treated
mice were examined. OCA-1 tumor bearing mice were prepared as previously
S8
3
described. When tumor volume reached 500 mm 9animals were injected with either
paclitaxel (80 mg/kg) or PG-TXL (I 60 mg equivalent paclitaxel/kg). At different
times ranging from 0 to 144 h after treatment, tumors were histologically analyzed to
quantify mitotic and apoptotic activity according to Milas et al. (199S). The mice
were killed by cervical dislocation and the tumors were immediately excised and
placed in neutral-buffered formalin. The tissues were then processed and stained with
hernatoxylin and eosin. Both mitosis and. . .

The changes observed in the paclitaxel-treated mice were qualitatively similar
5 to those previously described (Milas et al., 1995). The tumor cells showed marked
nuclear fragmentation with formation of apoptotic bodies, which was especially
marked on day I (FIG. 13). Viable tumor cell clumps with normal mitoses were still
present in these tumors by 144 h, indicating that these tumors would eventually
regrow. Treatment with. PG-TXL only resulted in a mild increase in mitotically
arrested cells and apoptotic cells, presumably due to the small amount of free
paclitaxel released from PG-TXL (FIG. 13). By 96 h, tumors from PG-TXL-treated
mice developed extensive edema and necrosis, and only a small rim of

viable tumor
cells remained. By 144 h, the residual tumor clumps as
compared to controls were
comprised of cells that were larger, more pleornorphic, and that
displayed less mitotic
activity.

. . .

as a water-
soluble form of paclitaxel, it is now clear that the agent used to
solubilize paclitaxel,
contributes to the overall anti-tumor activity of this
remarkable new complex. These
data indicate that PG-TXL has an ability to produce cell death in a
manner. . .

. . .

poly-lysine will be blocked by reacting the modified polymer with acetic
anhydride. TXL, docetaxel, other taxiods, etopside, teniposide,
camptothecin,
epothilone or other anti-tumor drugs will be conjugated to the
resulting polymer
according to previously described procedures for the synthesis of
PG-TXL.

. . .

to glutamic acid-containing copolymer by removing the
benzyl protecting group (FIG. 15). TXL, docetaxel, other taxiods,
etopside,
teniposide, camptothecin, epothilone or other anti-tumor drugs
will be conjugated to
the resulting polymer according to previously described procedures for
the synthesis
of PG-TXL and PG-CPT.

. . .

Based on the in vitro and early animal work, it appears that this
compound is at least as active again st cancer as the
monomeric paclitaxel in
Cremophor and may have fewer side effects. Based on these observations,
this drug
will be studied in. . . dose
of PG-TXL which may be used in subsequent Phase II studies in patients.
Phase II
studies will be performed in several tumor types to determine
the activity of PG-TXL
in various cancers. One of ordinary skill in the art will
recognize that modifications in
administration, selection of animal models and dose regiments may. . .

Phase 11 Studies
Phase II studies of PG-TXL will be performed in several tumor
types. Each
study will be designed in a usual standard Phase 11 manner following
either Gahan's or
Simon's design. In brief, approximately 14 patients of a given
tumor type will be
treated initially, if there is no evidence of anti-cancer
activity in that tumor type then
further studies of PG-TXL in that tumor type will be aborted.
However, if at least one
I 0 patient has clinical benefit, defined as at least 50% decrease in
the sum of products of
perpendicular cross-sectional diameters of the tumors, then
the number of patients
with that tumor type treated with PG-TXL will be increased to
30. These studies will
allow us to define the activity of PG-TXL in various cancers

and refine the
information on the side effects of the drug. The tumor types
of special interest for
1 5 PG-TXL will be the ones which have shown good response to paclitaxel
and
docetaxel. This will include ovarian cancer, breast
cancer, and lung cancer. Studies
comparing poly-glutamic acid-paclitaxel to paclitaxel in tumors
showing response to
PG-TXL will be performed. Such studies are called Phase III studies.

Phase III Studies of PG Paclitaxel
Based on the activity of paclitaxel in ovarian cancer, breast
cancer, and lung
cancer these will be the tumor types in which PG-TXL
will be compared to paclitaxel.
. . .
Phase I studies
may be completed in another 6 to 9 months. Once these have been
completed, Phase
11 studies in various tumor types may take another 6 to 9
months. At that point, the
inventors will have a good idea of the efficacy. . . studies will
show enough clinical activity that abbreviated Phase III studies or no
Phase III studies WO 99/49901 PCTIUS99/06870
64
EXAMPLE 12
ENHANCEMENT OF TUMOR RADIORESPONSE OF A MURINE
OVARIAN CARCINOMA BY POLY(L-GLUTAMIC ACID)-PACLITAXEL
CONJUGATE
Introduction
The combination of chemotherapy and radiation therapy in the treatment
of a
variety of tumors has produced substantial improvement in
complete response and
survival rates (Rotman, 1992). Both in vitro and in vivo studies have
demonstrated
that paclitaxel can strongly enhance tumor radioresponse. In
animal studies, the
enhancement factors range from 1.2 to more than 2.0, depending on the
tumor type,
drug concentration, and dose scheduling. This study investigated the
radiosensitization effects of poly(L-glutamic acid)-paclitaxel (PG-TXL).

When tumors reached 8 nun in diameter, mice were randomly
divided into 12 groups
with each group consisting of 6-12 mice. Mice in. . .

Local gamma irradiation to the tumor was delivered from a 'Cs
irradiator at a dose
rate of 7 Gy per minute. Tumor growth delay was determined by
measuring three
orthogonal tumor diameters until tumors reached 14
mm in diameter.
. . .
cell killing and rapid repopulation of surviving cells. At higher doses,
PG-
TXL may have profound effects on population of cycling tumor
cells and/or on tumor
reoxygenation, resulting in significantly enhanced radiosensitization
effect.

Interestingly, when tumors were irradiated at 14 Gy prior to
treatment with PG-TXL at

120 mg eq. paclitaxel/kg, a superadditive effect with an enhancement. . .

TABLE7
Effect of PG-TXL on Radioresponse of Murine Ovarian OCa- I Tumor
Treatments Radiation Days for Absolute Normalized Enhance-
tumor to growth growth ment factors
(14 GY) grow from delay in delay (95% C.L)c
8-14 mm daySa (mean±SD)b
(mean±SD)
Saline No 17.2 2.2
PG-TXL 47. . . eq/kg
14 Gy radia- Yes 37.9 6.1 20.7
tion alone
SUBSTITUTE SHEET (RULE 26)
TABLE7
Effect of PG-TXL on Radioresponse of Murine Ovarian OCa- I Tumor
Treatments Radiation Days for Absolute Normalized Enhance-
tumor to growth growth ment factors
(14 GY) grow from delay in delay (95% C.L)c
8-14 mm daySa (mean±SD)b
(mean±SD)
PG-TXL 47 Yes 35.3 ±. . . 100.5 88 ± 1.2 4.3 (4.1 -
mg eq/kg 4.4)e
a. Absolute growth delay is defined as the time in days for
tumors in treated
groups to grow from 8 to 14 mm minus the time in days for tumors
in saline
treated group to grow from 8 to 14 mm.

b. Normalized growth delay is defined as the time in days for
tumors to grow
from 8 to 14 mm in mice treated with the combination of PG-TXL and
radiation minus the time in days for tumors to grow from 8 to
14 mm in mice
treated with PG-TXL alone.

C. Enhancement factors are obtained by dividing normalized tumor
growth delay
in mice treated with PG-TXL plus radiation by the absolute growth delay
in
mice treated with radiation alone.

e. Data based on tumors that had regrown on day 120.
Tumors in 2 out of 6 mice
for both groups were still not measurable on day 120.
. . .
with
radiotherapy may be effectively used either before or after irradiation
to enhance
radiosensitization. These results further suggest that conjugation of
radiosensitizers
and anti-tumor drugs to water-soluble polymeric carriers may
offer enhanced
radiosensitization effect. In light of the present disclosures, one of
ordinary skill in
the. . . patient by patient basis, taking into
account, for example, factors such as the weight and age of the patient,
the type of
tumor being treated, the severity of the disease condition,
previous and/or concurrent
therapeutic interventions, the manner of administration and the like,
which. . .

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CLMEN I A method of enhancing the response of a tumor to
irradiation, comprising:
a) administering to a patient in need of such therapy a radiosensitizing
amount of a pharmaceutical composition comprising paclitaxel,. . .
docetaxel, eptopside,
teniposide, camptothecin or epothilone con ugated to a water solub4e
polyamino acid
polymer and a pharmaceutically acceptable carrier;
b) irradiating said tumor;
wherein said conjugated paclitaxel or docetaxel have increased water
solubility,
1 0 efficacy and accumulation within a tumor compared with the
corresponding
unconjugated drugs.

8 The method of claim 1, wherein step (b) is carried out by
administering
gamma irradiation to said tumor.

12 The method of claim 9, wherein said cancer is breast
cancer, ovarian cancer,
malignant melanoma, lung cancer, gastric cancer,
prostate cancer, colon cancer, head
and neck cancer; leukemia or Kaposi's sarcoma.
1 5 13. A method of treating cancer comprising:
a) administering to a patient in need of such therapy a radiosensitizing
amount of a pharmaceutical composition comprising paclitaxel, docetaxel,
eptopside,
teniposide, camptothecin or epothilone conjugated to a polyglutamic acid
polymer and
a pharmaceutically acceptable carrier; and
b) irradiating said tumor.

=>

---Logging off of STN---

=>
Executing the logoff script...

=> LOG Y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	28.96	29.17

STN INTERNATIONAL LOGOFF AT 11:19:02 ON 28 AUG 2006